Variability of *Helianthus maximiliani* Schrader revealed by RAPD analysis

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Summary

Variability of Schrader revealed by RAPD analysis

Intraspecies variability of *Helianthus mamiliani,* as well as its relations with two other wild species and cultivated sunflower, was determined using RAPD (randomly amplified polymorphic DNA) analysis. Ten 10-base primers were used for the amplification. RAPD products were analysed by electrophoresis in 1.6% agarose gel. Significant variability within *H. mamiliani* was revealed. Considerable difference of several accessions compared with the rest raised the question about their taxonomic status. Dendrograms obtained on the basis of primers C15 and E05, which were in accordance with existing taxonomy, confirmed the usefulness of RAPD analysis for taxonomic studies.

Key words: *Helianthus mamiliani*, intraspecies variability, RAPD analysis, sunflower

Résumé

Mise en évidence de la variabilité de *Helianthus mamiliani* Schrader par analyse de RAPD

La variabilité intraspécifique d'*Helianthus mamiliani,* ainsi que ses relations avec deux autres espèces sauvages et cultivées de tournesol, ont été déterminées par analyse de RAPD (randomly amplified polymorphic DNA). Des amorces de 10 nucléotides ont été utilisées pour l'amplification. Les produits de RAPD ont été analysés par électrophorèse sur gel d'agarose à 1,6 %. Une variabilité significative au sein de *H. mamiliani* a été mise en évidence. Plusieurs accessions présentent des différences considérables par rapport à d'autres, ce qui soulève la question de leur position taxonomique. Les dendrogrammes obtenus avec les amorces C15 et E05, qui correspondent aux données taxonomiques actuelles, confirment l'utilité de l'analyse de RAPD dans les études taxonomiques.

Resumen

Variabilidad de *Helianthus mamiliani* Schrader revelada por análisis de APAA

La variabilidad intraespecífica de *Helianthus mamiliani,* así como sus relaciones con otras dos especies silvestres y cultivadas de girasol, se determinó mediante análisis del APAA (ADN polimórfico amplificado al azar). Se utilizaron diez activadores de base 10 para la amplificación. Los productos del APAA se analizaron por electrofóresis en gel de agar al 1,6%. Se encontró una notable variabilidad dentro del *H. mamiliani* . La diferencia considerable de varias accesiones en comparación con el resto planteó la cuestión de su estatus taxonómico. Dendrogramas obtenidos sobre la base de activadores C15 y E05, que concordaban con la taxonomía actual, confirmaron la utilidad del análisis de APAA para los estudios taxonómicos.

Introduction

Helianthus maximiliani Schrader belongs to the section Divarcati, series Gigantei of the genus *Helianthus* (Rogers et al. 1982). This species is very interesting from the agronomic aspect, because it was found that some of its populations (Skoric and Rajcan 1992) and clones (Henn et al. 1997) are resistant to *Sclerotinia sclerotiorum* Lib. de Bary.

H. maximiliani is fairly easily to distinguish species throughout its range but, as is true for most sunflowers, the important diagnostic characters of the leaves are so modified in some individuals that identification becomes uncertain (Heiser et al. 1969). Similarly as the leaf characters, most morphological parameters used for species identification may vary depending on environmental conditions. That is why the data obtained using DNA polymorphisms for determination of inter- and intraspecies variability could be considered more reliable, as there is no effect of the environment.

RAPDs (random amplified polymorphic DNAs) are now commonly used for estimating genetic relationships among closely related populations or species of plants (Rieseberg 1996). This technology (Williams et al, 1990) is fast yet simple to perform and permits the rapid screening of a large number of samples.

The objective of our work was to determine intraspecies variability of *H. maximiliani* using RAPD analysis, and to determine its relations with two other wild species as well as cultivated sunflower.

Material and methods

An inbred line of cultivated sunflower CMS_3-8A and accession 1631 of *H. maximiliani* were provided by the Institute of Field and Vegetable Crops, Novi Sad, Yugoslavia. Twenty-two accessions of *H. maximiliani* as well as one population of *H. mollis* and wild *H. annuus* were from the wild sunflower collection of INRA, Montpellier, France.

DNA was isolated from a mixture of 1 g of frozen leaf tissue from 10 plants belonging to the same accession, according to the method described by Gentzbittel et al. (1994). Purified DNA was quantified on 1% agarose gel with λDNA as the reference and was adjusted to 6 ng µl–1 for polymerase chain reaction (PCR) amplification.

Primers for amplification were chosen based on the results of Sossey-Alaoui et al. (1998). Ten of the primers from which unique markers of perennials present in *H. maximiliani* were obtained were chosen for our study. PCR amplification was performed in a 25-µl reaction volume containing 2.5 µl reaction buffer (Appligene), 0.2 mM dNTP, 0.5 µM primer, 30 ng DNA and 1.2 units *Taq* (Appligene). Amplifications were performed in a Perkin-Elmer GeneAmp PCR System 9700 in temperature conditions as described by Sossey-Alaoui et al. (1998). RAPD products were analysed by electrophoresis in 1.6% agarose gel. Presence of fragment was noted as 1, whereas absence was noted as 0.

The data obtained were used for grouping the accessions. Analytical precision is increased by introducing a large number of qualitative and quantitative parameters, which is enabled by the method of hierarchical cluster analysis. This method was prepared as an analytical tool for investigating data where the numbers of categories in each clarification are large (Lin 1982). For estimating the differences between the groups, euclidian units were used, where distance x, y=$[E_i(x_i–y_i)^2]^{112}$. Groups of accessions were separated using single linkage method, e.g. nearest neighbour.

Data processing and the construction of dendograms were done using SATATISTICA 5.0 for Windows (1995).

Results and discussion

In total, 189 RAPD fragments were analysed. The greatest number of fragments was obtained using C02 and All primers (25) and the lowest using primer E05 (5). The size of fragments ranged from 100 to 2300 kb.

On the dendrogram obtained on the basis of all ten primers, two main groups can be observed (Figure 1). *H. maximiliani* accession 569 formed a separate group. Except for this, the dendrogram revealed intraspecies variability of *H. maximiliani* and was in accordance with phylogenetic relations of tested species.

The inbred line of cultivated sunflower grouped with wild *H. annuus,* while *H. mollis* formed a separate group and was more closely related to *H. maximiliani*. This is in accordance with the sunflower systematics suggested by Rogers et al (1969) that groups these two species in the same section.

Within the species, several populations grouped separately, with population 1631 being the most different. Similar results were obtained by Miljanovic et al (2000), who analysed quantitative, qualitative and biological traits of 15 populations of this species. The greatest distance between population 1631 and other accessions of the species was observed on dendrogram obtained by E03 primer fragments analysis (Figure 2).

Interesting results were obtained by analysing fragments obtained with primer C15 and E05. These primers revealed interspecies variability, and almost no polymorphism within *H. maximiliani* (Figures 3 and 4). As in the work of Sossey-Alaoui et al (1998), fragment C15–650 appeared neither in *H. mollis* nor in both *H. annuus* therefore enabling their separation from *H. maximiliani.* Common markers (C15–300, –350, –2000), according to the same authors, either did not appear or were only detected in wild *H. annuus.* Fragment E05–900 appeared only in perennial species. This is in accordance with the results of Sossey-Alaoui et al (1998) who found this fragment to be a unique marker for perennials. The same authors found E05–1600 fragment a common marker for annuals and perennials, as was the case in our investigation. Out of 189 analysed fragments 5 were unique for cultivated sunflower, 9 for wild *H. annuus,* 5 for *H. mollis* and 5 for all *H. maximiliani* accessions. Some fragments appeared only in annual species and others

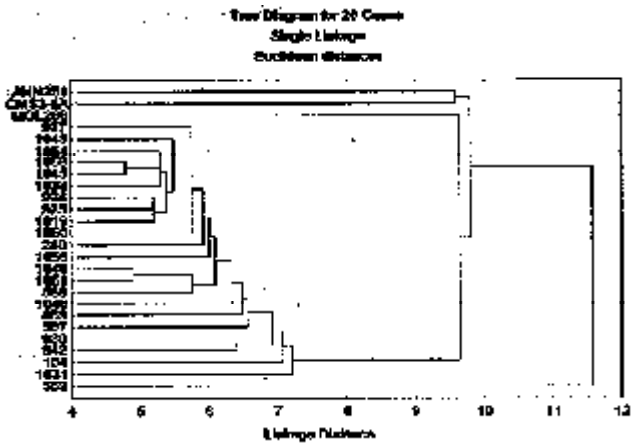

Figure 1. Tree diagram for all primers.

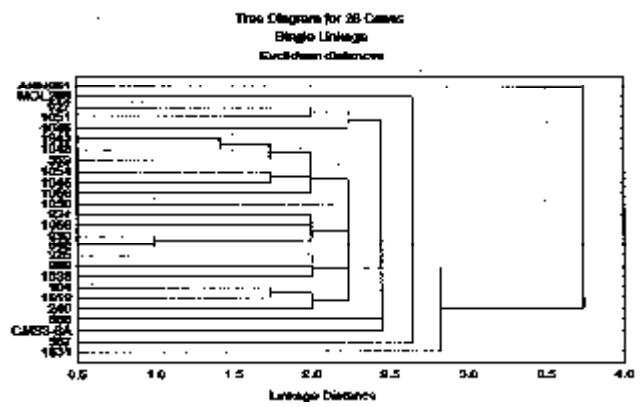

Figure 2. Tree diagram for E03 primer.

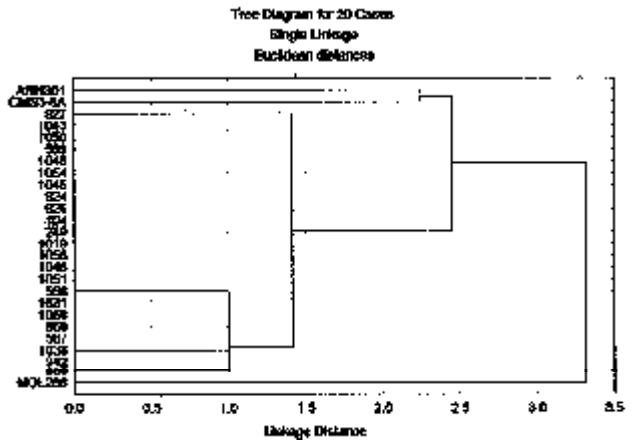

Figure 3. Tree diagram for C15 primer.

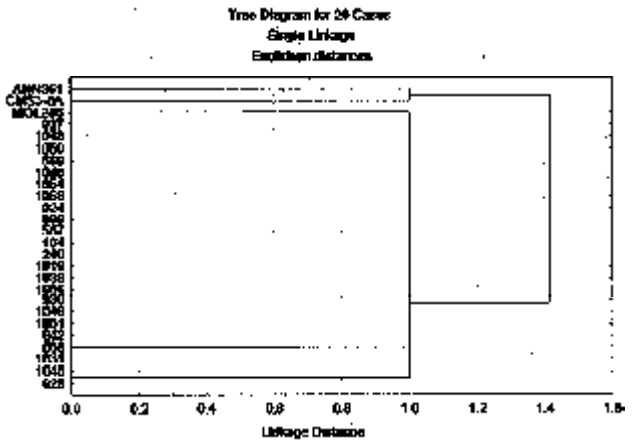

Figure 4. Tree diagram for E05 primer.

appeared only in perennial species, thus being candidates for markers of annuals or perennials. Fragments All–1800, C04–1500, C15–1300 and –2000, and E05–1200 were detected only in annual genotypes i.e. inbred line CMS_3-8A and wild *H. annuus.* Seven fragments (All 1–950, C04–1 100, C14–850, C–550 and –750, C19–2100, and E05–800) were present only in perennial species i.e. *H. mollis* and *H. maximiliani.* Besides E05–1600, only one other fragment was common to all tested species and genotypes: C15–900.

Conclusions

As in previous investigations based on morphology, significant variability within *H. maximiliani* was revealed by RAPD analysis. Considerable difference of several accessions compared with the rest raises the question about their taxonomic status. This is especially true for populations 569 and 1631.

Dendrograms obtained on the basis of primers C15 and E05, which were in accordance to existing taxonomy, confirmed the usefulness of RAPD analysis for taxonomic studies. Detected unique or common fragments could be included in further studies of variability within the genus *Helianthus* using RAPD analysis.

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